Reference: *Biol. Bull.*, **146**: 116–136. (February, 1974)

THE CHOROID RETE MIRABILE OF THE FISH EYE. I. OXYGEN SECRETION AND STRUCTURE: COMPARISON WITH THE SWIMBLADDER RETE MIRABILE [1]

JONATHAN B. WITTENBERG [2] AND BEATRICE A. WITTENBERG

The Department of Physiology, Albert Einstein College of Medicine, New York, New York 10461 and The Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Vascular counter-current systems in which the vessels are of capillary dimensions have been described for the mammalian kidney, the swimbladder of teleost fish (Woodland, 1911; Harden Jones and Marshall, 1953), and the choroid layer subtending the retina of the fish eye. Albers (1806) was the first to show that the large, horseshoe-shaped body in the choroidal layer of the eye of fish was neither a muscle nor a gland as was then supposed but an aggregation of small blood vessels. These vessels originate from a branch of the ophthalmic artery and their outflow supplies the choriocapillaris, the dense capillary bed underlying the retina (Müller, 1839; Jones, 1838). Müller (1839) pointed out that all the blood to the choroidal vessels had first to pass through the pseudobranch. Allen (1905) injected the vessels of the choroidal circulation and later (1949) gave a detailed account of them. He pointed out that the blood flowing to the eye in the ophthalmic artery is oxygenated at the gills and, in many species, again comes in contact with sea water at the pseudobranch. Jones (1838) introduced the now preferred name for the choroidal vascular structure—the choroid rete mirabile.

Barnett (1951) gives by far the most detailed description of the choroidal circulation. It is based on dissection of injected preparations and on serial sections of whole eyeballs or whole heads. Johannes Müller (1839), Richard Owen (1836) and T. Wharton Jones (1838) had each recognized that the arterial and venous capillaries making up the choroid rete mirabile are arrayed in parallel. It remained for Barnett (1951) to realize that the arterial and venous blood streams flow counter-current one to another in the capillaries of the choroid rete mirabile. In addition to the choroid rete mirabile, Barnett (1951) describes a similar, smaller structure, the lentiform body, also supplying blood to the choriocapillaris.

It appeared to us that the pigment cell epithelial layer of the retina is at least formally analogous to the gas gland of the swimbladder (Wittenberg and Wittenberg, 1962). These relations are diagrammed in Figure 1. In each instance a capillary counter-current organ, a rete mirabile, supplies arterial blood to a capillary network, underlying an epithelial layer, respectively the pigment cell layer of the retina and the gas gland of the swimbladder, and in turn receives the venous outflow from these capillaries. The structural resemblance between the swimbladder

[1] This investigation was supported in part by Research Grants G20020 and GB 20981 from the United States National Science Foundation and by a United States Public Health Research Grant, H3719, from the National Heart Institute.

[2] Research Career Program Awardee 01-HL6–733 of the United States Public Health Service, National Heart and Lung Institute.

and the choroid retia suggest to us that their functions may be similar, and that the choroid rete mirabile-pigment cell layer complex may serve to create a large pressure of dissolved oxygen behind the retina, providing a pressure for diffusion to supply the vigorous oxygen demand of the avascular retina. We searched for and found large oxygen pressures in the vitreous humor, near the retina of the fish eye (Wittenberg and Wittenberg, 1962), an observation confirmed by Fairbanks, Hoffert and Fromm (1969).

In the present communication we present a more full account of these findings, and examine quantitatively the way in which the structure of the choroid rete mirabile is adapted to provide a large flow of oxygen toward the retina. We describe the structure of the choroid rete mirabile of the holostean, *Amia calva,* and amplify Barnett's (1951) description of the choroid rete of teleosts. Elsewhere

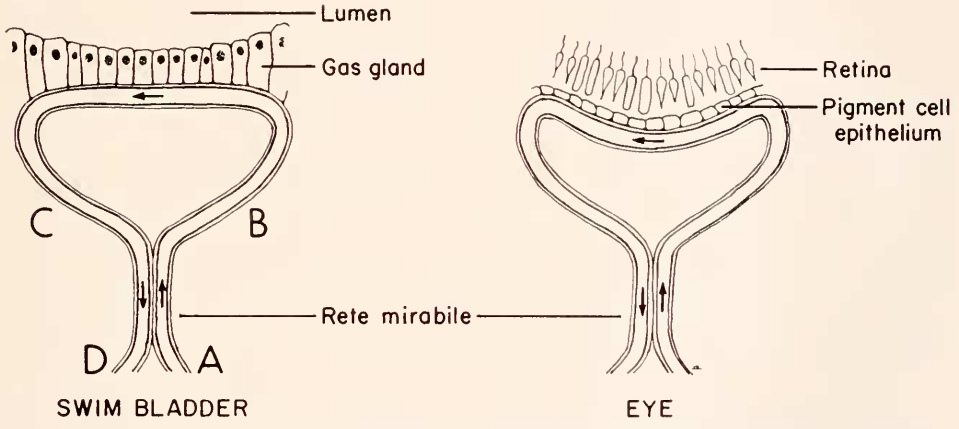

FIGURE 1. Diagram comparing the oxygen-secreting complexes of the eye and the swimbladder of fish.

the distribution of the choroid rete mirabile among fishes and its relation to the pseudobranch is considered (Wittenberg and Haedrich, 1974).

MATERIALS AND METHODS

Animals

Most of the marine fish used in this study were captured in a fish trap maintained by the Marine Biological Laboratories, Woods Hole, Massachusetts. Some were caught in pots or on hand lines. Cod were the gift of Mr. Charles Wheeler, Bureau of Fisheries, Woods Hole, Massachusetts. Specimens of *Amia* (*Amia calva*) (used for anatomical studies) were the gift of Mr. William A. Lemberger, Oshkosh, Wisconsin. Specimens of *Amia* and gars (Lepisostidae) (used for measurement of ocular pO_2) were captured by Dr. D. Eugene Copeland, Tulane University, New Orleans. Anatomical studies were made of the eyes of cod (*Gadus morhua*), bluefish (*Pomatomus saltatrix*) and of the holostean, *Amia calva*.

Histology and injection preparations

Histological sections were prepared from eyes fixed in Bouin's solution. Blood vessels were injected through the ophthalmic artery or vein with Indian ink in 7% gelatin, with yellow lead chromate in 7% gelatin, or with neoprene latex. Injected preparations were fixed in acid formaldehyde (formalin, 10 volumes, glacial acetic acid, 5 volumes, water to 100 volumes) and were transferred to 70% ethanol for dissection or were cleared and dissected in benzyl benzoate.

Measurements of dimensions of the retia or of intraretinal distances were made with a calibrated ocular micrometer. All measurements are referred to tissue fixed in Bouin's solution and measured in 70% ethanol. Measurements of histological sections are corrected for 22% linear shrinkage. Each set of data given in Table IV is for an individual animal. An additional specimen of each species was examined with similar results. The eels were of average size, 1–2 lb; the conger was of modest size, about 10 lb; the swordfish weighed 160 lb dressed, which is not large; and bluefin tuna weighed about 150 lb.

Oxygen pressure in the eye

Oxygen pressure was measured with an oxygen sensing electrode mounted in the tip of an 18-gauge hypodermic needle (Beckman Spinco Division, No. 161–950 Oxygen micro-electrode). The electrode current was measured with a Keithly model 600A, battery-operated electrometer, or with a Beckman model 160 oxygen analyzer. Calibration was with air equilibrated water and with a solution of zero oxygen pressure (buffered 5% glucose containing glucose oxidase and catalase).

The electrode was introduced into the front of the eye passing between the lens and iris into the vitreous humor. Most measurements were made with the sensing tip of the electrode in the vitreous humor immediately in front of the retina.

Measurements of tissue oxygen pressure made with oxygen electrodes are subject to a number of errors due to inhomogeneity of the tissue and occlusion of capillary blood flow at the electrode tip. The measurements reported here are free from these difficulties. The readings were stable with time and, since the diffusion coefficient of oxygen in a material as fluid as the vitreous humor is scarcely different from that in water, should correspond closely to the true oxygen pressure. If the tip of the electrode chanced to be pushed against the retina, the apparent oxygen pressure dropped immediately, suggesting that the circulation was occluded locally. Further validation of the use of the oxygen electrode comes from the work of Jacobi and Driest (1965), who found, as expected, that the measured oxygen pressure of the vitreous of the rabbit eye increased as the electrode was moved closer to the source of the oxygen at the vascular retina.

Measurements on marine fish were made on living fish with a current of sea water passing over the gills. Measurements on the freshwater fish, gars and *Amia*, were made on living animals restrained and submerged in the water of the bayou.

Gars (spotted gar, *Lepisosteus oculatus* and alligator gar, *L. spatula*) and *Amia* were seined from a shallow bayou near Lake Penchant in the Mississippi River delta. Measurements were made immediately as the fish were brought to the surface so that measurements on gars and on *Amia* are interspersed. During the

TABLE I

Oxygen partial pressure in the eyes of fish

Species		No. of animals	Oxygen pressure Average (range) (mm mercury)	Rete
Marine fish				
Sting ray	*Dasyatus centroura*	2	16 (7–34)	Absent
Skate	*Raja ocellata*	3	10 (6–17)	Absent
Smooth dogfish	*Mustelus canis*	3	11 (9–11)	Absent
Eel	*Anguilla rostrata*	2	18 (9–23)	Absent
Conger	*Conger oceanica*	4	8 (2–12)	Absent
Toadfish	*Opsanus tau*	4	18 (6–28)	Minute
Goosefish	*Lophius americanus*	4	93 (39–146)	Minute
Sea bass	*Centropristes striatus*	2	161 (130–180)	Small
Tautog	*Tautoga onitis*	1	210	Small
Sea robin	*Prionotus carolinus*	3	468 (280–860)	Large
Fluke	*Paralichthys dentatus*	1	255	Large
Puffer	*Sphaeroides maculatus*	2	462 (365–575)	Large
Scup, porgy	*Stenotomus versicolor*	7	484 (272–770)	Large
Menhaden	*Brevoortia tyrannus*	2	255 (240–287)	Large
Goggle eye jack	*Trachurops crumenophthalmus*	4	416 (317–566)	Large
Cod	*Gadus morhua*	3	820 (575–1180)	Large
Bluefish	*Pomatomus saltatrix*	4	454 (240–820)	Very large
Remora	*Echeneis naucrates*	3	775 (435–1320)	Very large
Freshwater fish				
Trout	*Salmo gairdneri*	16	445 ± 68.5*	Large
Gar	*Lepisosteus* sp.	5	90 (72–145)	Absent
Bowfin	*Amia calva*	14	200–650**	Large

* Data of Fairbanks, Hoffert and Fromm (1969).

** See text and Table II.

course of the day the temperature of the water near the surface rose from about 22° C to about 33° C. The bottom water, from which the fish were taken, remained cool. This can introduce an artifact in the determination of pO_2, since at constant oxygen content, pO_2 increases with the temperature of the solution (in this instance the vitreous humor). The maximal effect of this artifact, for the temperature interval 22–33° C, is to increase the apparent pO_2 by 20%. An internal control of this artifact is comparison of the pO_2 in the eye of *Amia* with that of gars, which do not have an elevated oxygen pressure in the eye, measured in the same series of experiments. A second source of error is the small size of the eye of *Amia* relative to the electrode (which was the smallest available at the time, 1963, the experiments were done). Many times the apparent initial pO_2 in the eyes of *Amia* was large but drifted downward before a stable reading was obtained. This effect was not seen with gars, in which the eye is larger. The data are presented in Table II. Only stable readings were recorded in the table; they are not corrected for the possible temperature artifact.

Measurements on warm-blooded animals were made in a 40° C room, so that the electrode temperature differed only slightly from that of the animal. Cats were anesthetized with nembutal, 30 mg per kilogram, administered intraperitoneally. Rabbits were anesthetized with urethane, delivered slowly into the ear vein

until deep anesthesia was achieved. Birds were anesthetized with ether. The animals were sacrificed without recovery from anesthesia.

Results and Discussion

Very large pressures of oxygen were found in the eyes of some fish, Table I. A variety of bottom-living marine fishes, sea robins, fluke, puffer and scup and two pelagic fishes, jack and menhaden, exhibited oxygen pressures in the vitreous humor ranging from 250 to 800 mm Hg. The oxygen pressure in the eye of trout living in fresh water (data of Fairbanks *et al.*, 1969) falls within this range. These pressures should be compared to the oxygen pressure of arterial blood, which is presumably close to that of the ambient water, 155 mm Hg, and in trout is 85 mm

Table II

Oxygen pressure in the eyes of individual gars and Amia.

Species	Oxygen pressure	
	Right eye	Left eye
	mm Hg	
Spotted gar *Lepisosteus oculatus*	87	145
	80	102
	—	75
Alligator gar *Lepisosteus spatula*	72	80
	80	—
Bowfin *Amia calva*	204	—
	167	180
	220	—
	62	1210
	440	—
	400	136
	240	235
	100	180
	390	300
	—	220
	—	590
	104	770
	845	—
	—	115

Hg (Stevens and Randall, 1967). Three species, cod, bluefish and remora, deserve special comment. The choroid rete of the last two of these fast-swimming, sight dependent, predaceous animals is so greatly developed that the rear of the eyeball bulges asymmetrically to accommodate it. The rete of the third predator, cod, is large but symmetric. The bluefish tolerated handling poorly, and the measurements of oxygen pressure in the eye of this species were at best erratic. The remora and the cod, by contrast, are tough and resistant to laboratory insult. In two individual remora and one cod we measured oxygen pressures of 1000, 1300 and 1180 mm Hg respectively, pressures greatly in excess of one atmosphere (760 mm Hg).

We were led to perform these experiments by the postulate that the choroid rete mirabile is concerned with oxygen transport. This postulate may be evaluated

by comparison of species in which the rete is small or lacking with those in which it is large. We have examined six species which lack choroid retia. Of these two, eel and conger are teleosts; three, sting ray, skate and smooth dogfish, are elasmobranchs; and one, the gar, is a holostean. All exhibit low oxygen pressures (10–30 mm Hg) in the vitreous humor. The goosefish and toadfish depend in part on chemical or tactile senses to find their food. The choroid rete of these species is minute, and the oxygen pressure in the vitreous is correspondingly low, scarcely exceeding the probably venous oxygen pressure. Two bottom-living teleosts, sea bass and tautog, have relatively small choroid retia and exhibit oxygen pressures in the vitreous not too different from air. Teleosts with larger choroid retia exhibit correspondingly larger oxygen pressures in the vitreous. The observed correlation between the measured oxygen pressure and the extent to which the choroid rete is developed suggests that the rete plays an essential part in establishing the large oxygen pressure at the retina.

TABLE III

Oxygen pressure in eyes of some vertebrates

Species	Number of animals	Oxygen pressure Average (range)
		mm Hg
Bullfrog *Rana catesbiana*	3	41 (33–59)
Alligator *Alligator mississipiensis*	1	42
Turtle *Pseudemys floridana*	3	41 (37–47)
Turtle *Graptemys pseudogeographica*	2	29 (25–34)
Rabbit	4	16 (7–31)
Rabbit	–	— (17–33)*
Cat	3	19 (15–22)
Cat	–	19**
Pigeon	5	50 (32–64)
Chicken	3	48 (40–53)
Duck	1	44

* Data of Jacobi and Driest (1965).
** Data of Alm and Bill (1972).

The choroid rete mirabile of the holostean, *Amia calva,* the only non-teleost found to have a rete (Wittenberg and Haedrich, 1974), resembles that of teleosts. The rete of *Amia* may represent an independent evolutionary development convergent with that of teleosts; or teleosts and *Amia* may share an immediate common ancestor. This at present is a matter of debate (Nelson, 1969a, 1969b). For this reason it was of particular interest to establish whether the oxygen pressure in the eye of *Amia* is elevated. This measurement was made with some difficulty and an average normal value cannot be given. Nonetheless the data assembled in Table II leave no doubt that a large oxygen pressure obtains in the *Amia* eye. We conclude that the choroid retia of *Amia* and teleosts serve the same function—to transport oxygen toward the retina.

Maintenance of a large oxygen pressure near the retina appears to be particular to teleosts and *Amia*. Data on the oxygen pressures in the eye of other vertebrates,

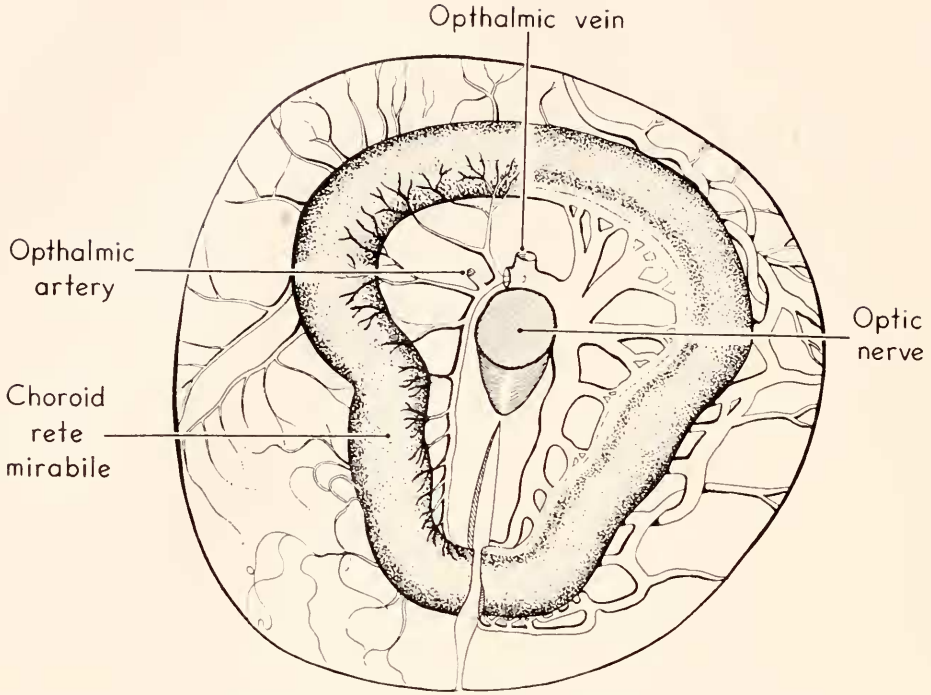

FIGURE 2. Choroid rete mirabile of the cod. Right eye viewed from inside the orbit. The right half shows the ophthalmic venous sinus and its drainage. On the left, the venous structures are dissected away to show the arteries.

collected in Table III, show only low pressures close to those expected of venous blood.

We now describe the anatomical relations of the teleost choroid rete mirabile. The choroid rete mirabile lies within the eyeball in the choroidal layer exterior to the retina and separated from the sclera by membranes which may, in different species, be laden with fat, or thin and unpigmented, or highly reflective and silvery, or intensely black. Silvery or black membranes may lie between the rete and the choriocapillaris (Denton, Liddicoat and Taylor, 1970). The rete itself is not pigmented. It has the shape of a horseshoe, with the open end oriented ventrally (Fig. 2) or, particularly in species in which the rete is large, anteriorly (Fig. 3). In species such as the bluefish in which the rete is large, the eye may lose its simple oblate shape and the sclera may bulge conspicuously to accommodate the bulk of the rete. There is no strong attachment of the rete to structures other than blood vessels in the interior of the eye. Figure 2 portrays the disposition of the choroid rete mirabile within the eye of a codfish viewed from inside the orbit. In this view the counter-current capillaries and small vessels are arrayed nearly normal to the plane of the paper and are hidden from view by the overlying ophthalmic venous sinus.

The ophthalmic artery pierces the sclera posterior, dorsal and close to the optic nerve, and passes into the ophthalmic venous sinus that forms the inner

border of the choroid rete. Therein it divides into two branches, each supplying a limb of the rete. These branches may ramify into subsidiary branches running toward the rete proper as in the cod (Fig. 2); or they may be closely applied to the base of the capillaries and there arborize into stubby twigs at right angles to the main branch, as in bluefish (Fig.3). In either case, the arterial capillaries of the rete mirabile proper arise abruptly and almost directly from the thick-walled arteries (Fig. 4). The arteries run submerged in the ophthalmic venous sinus (Figs. 3 and 4).

The arterial retial capillaries coalesce to form a bed of intermediate arterial vessels which in turn coalesce into the arterial vessels supplying the choriocapillaris. These intermediate vessels are by no means a negligible part of the structure. In Figure 4 we attempt to show them in true proportion; their aggregate volume must exceed considerably the aggregate volume of the capillary vessels of the rete proper. In some larger fishes, most conspicuously in the gempylid, *Lepidocybium flavobrunneum*, but also in many others, the rete proper is only a thin sheet capping an enormous, tangled but roughly parallel array of coarse intermediate vessels filling much of the back of the eye, suggesting a mass of spaghetti.

The efferent arteries break up into fine arteries in the vascular layer underlying the retina (Fig. 4), and these arteries in turn supply the capillaries of the choriocapillaris. Ordinarily these vessels are as small as they are portrayed in Figure

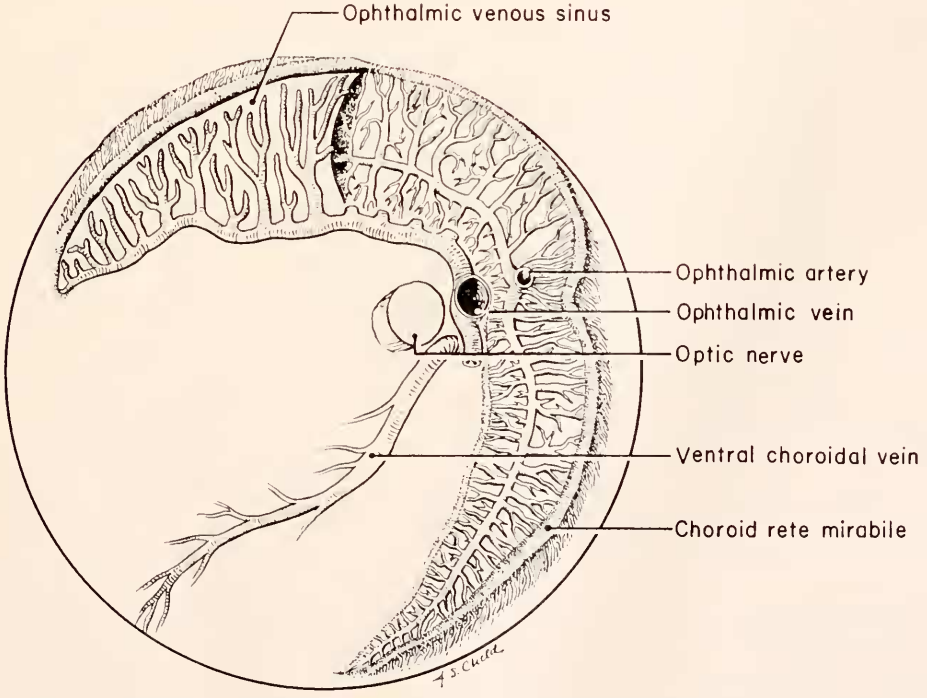

FIGURE 3. Choroid rete mirable of the bluefish. Right eye viewed from inside the orbit. The ophthalmic venous sinus is shown largely removed to display the underlying arteries.

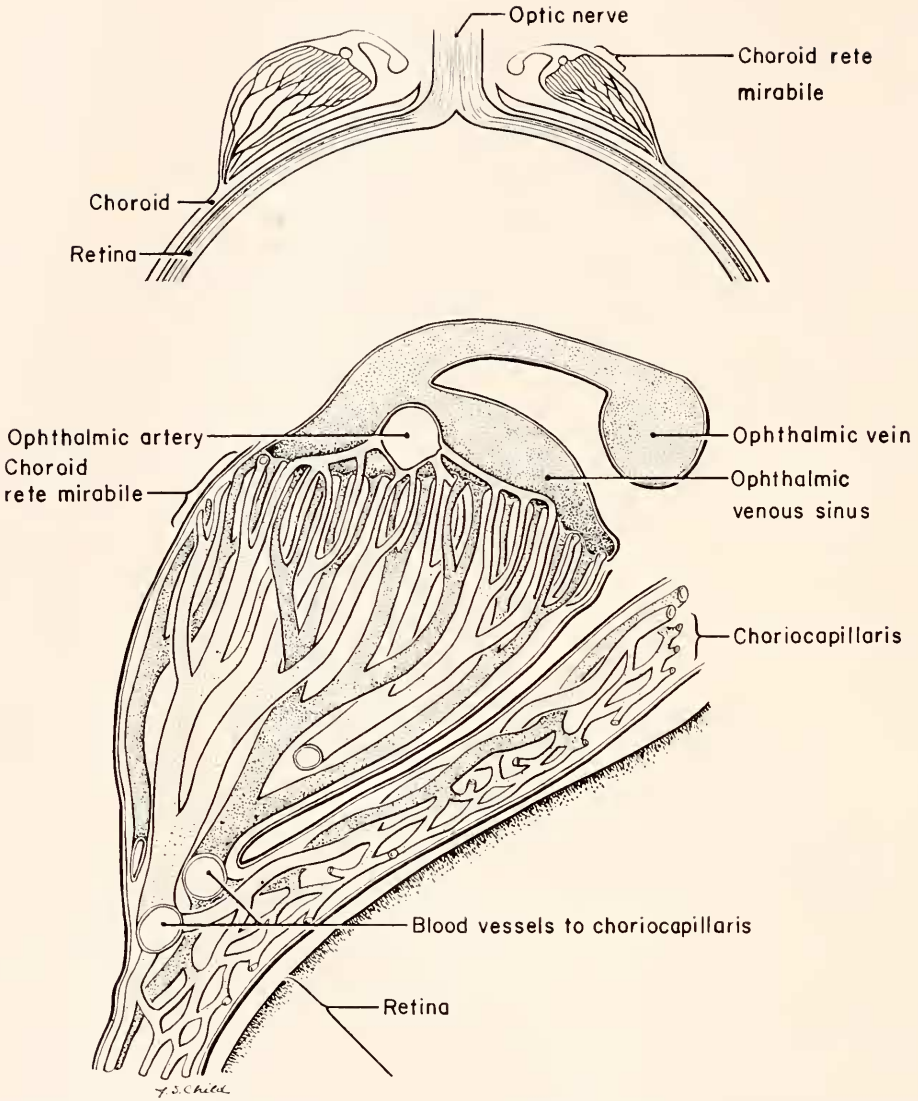

FIGURE 4. Semidiagrammatic representation of the choroid rete mirabile of the bluefish seen in longitudinal section. For clarity the size of the capillaries is exaggerated. The insert places the choroid rete in relation to the optic nerve and retina.

4. In the swordfish (*Xiphias gladius*), these vessels elaborate to form a hexagonal mesh of confluent vessels of rather large caliber (approximately 0.5 mm diameter) underlying the entire choriocapillaris and supplying it with blood by way of fine sprigs arising directly from the coarse vessels. The volume of blood contained in these vessels of the swordfish and in the intermediate arterial vessels noted in *Lepidocybium* must be large. Possibly it serves as a mobile reservoir of heat,

generated metabolically and conserved within the eye by the action of the choroid rete mirabile. In some species a carotid rete such as that described for the tuna by Linthicum and Carey (1972) may act in consort with the choroid rete as a barrier to heat loss. Some larger fish do maintain a high retinal temperature (Linthicum and Carey, 1972). A mobile reservoir of blood may help to dissipate the thermal gradients which Linthicum and Carey show to arise from unequal cooling of the eye. Consistent with this hypothesis is the fact that the vital structures of swordfish and gempylid eyes are blanketed by a thick layer of fat between the choroid and sclera.

The venous outflow from the choriocapillaris returns through small vessels to the venous capillaries of the rete mirabile proper. These empty without intervention of venules, directly into the ophthalmic venous sinus (Fig. 4). The ophthalmic vein drains the venous sinus and emerges through the sclera close to the optic nerve and separately from the ophthalmic artery.

TABLE IV

Dimensions of retia mirabilia

	Area supplied		Rete dimensions		Arterial capillaries		Venous capillaries	
	Chorio-capillaris and retina	Gas gland	Capillary length	Cross section area	Number	Diameter	Number	Diameter
	cm^2	cm^2	cm	cm^2	millions	micrometers	millions	micrometers
Choroid rete								
Tuna	31		0.18	8.4	7.94	10.5	8.14	23.2
Swordfish	44		0.22	5.6	7.21	15	3.88	18
Average of 86 teleosts			0.14					
Swimbladder rete								
Eel		43	0.77	0.86	0.67	21.6	0.356	33.7
Conger		30	0.49	0.26	0.13	25.2	0.066	51.0
Bluefish			0.36					

The rete mirabile proper consists of many thousands of closely arrayed and parallel arterial and venous capillaries in which the afferent and efferent blood streams flow counter-current one to another. Partially injected preparations of the choroid rete mirabile of the bluefish, in which only arterial vessels contain the injection mass, identify the thick-walled capillaries of the rete as arterial and thin-walled capillaries as venous. Close-packing of the capillaries to achieve optimal exchange of materials is demonstrated by their orderly array seen in cross-section.

The capillaries may be arrayed as in a checkerboard, for instance in the tuna eye, in which case each venous capillary is surrounded by four arterial capillaries and the numbers of arterial and venous vessels are equal (Table IV). Or, as in the swordfish eye, the capillaries may be in hexagonal array, in which case each venous capillary is surrounded by six arterial capillaries and the number of arterial vessels is twice the number of venous vessels (Table IV). These arrangements of capillaries are illustrated diagrammatically in Figure 5. Less regular arrays in which each capillary tends to be surrounded by five neighbors are also en-

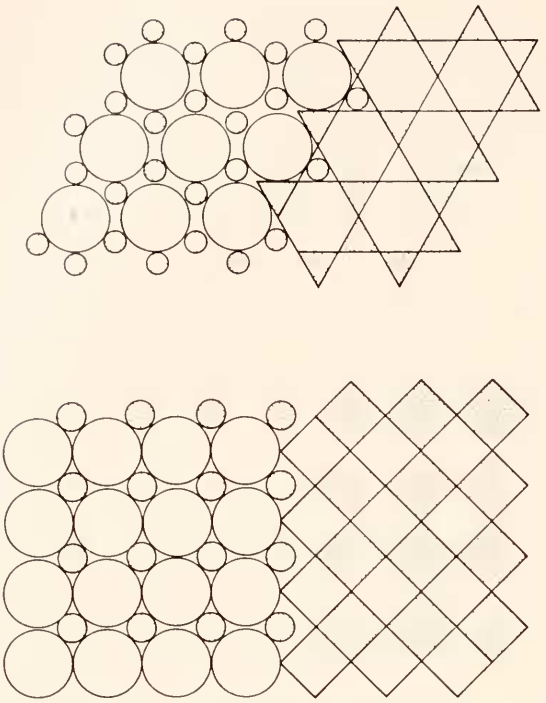

FIGURE 5. Diagram showing two ways in which arterial capillaries (stippled) and venous capillaries (white) may be arranged in a rete mirabile.

countered. We have not done serial sections, and cannot say whether these packing-patterns persist unchanged along the length of the rete.

An important aspect of retial structure, which must be taken into account in formulating any theory of the action of the counter-current system is that the choroid rete is not uniform along the length of the capillaries. In longitudinal sections of the rete of all fish, but particularly conspicuous in the rete of larger fish such as tuna or opah (lampridae, *Lampris regius*), the rete is seen clearly to be differentiated into proximal and distal portions of differing structure, with a sharp line of demarcation between them. This suggests that oxygen transfer by the choroid rete may be a two-stage process. The way in which the structures of the two parts of the rete differ is not easily resolved at the light microscope level. A study of the fine structure of the retial capillaries has been initiated in the laboratory of Copeland (*e.g.*, Wolley and Copeland, 1970) and we await their resolution of this problem.

We next describe the choroid rete mirabile of *Amia*. The appearance of this rete, viewed from inside the orbit, is portrayed in Figure 6. The sclera, covering membranes and the ophthalmic venous sinus have been dissected away. As in teleosts, the ophthalmic artery divides into two main branches, which arborize over the surface of the rete. The elegant and ordered symmetry of the teleost rete is lacking, and, in contrast to the teleost pattern, the retial capillaries arise from stubby

branches of the larger arteries (Fig. 6). Aside from these minor differences, we find no essential difference between the choroid retia mirabilia of *Amia* and of teleosts.

D. W. Fawcett, Harvard Medical School (personal communication) has made electron micrographs of the choroid rete of *Amia*. His drawings, based on these micrographs (Fig. 7), contrast the structure of the capillaries of the choroid rete of *Amia* with those of the swimbladder rete of the toadfish, *Opsanus tau*. (Fänge and Wittenberg, 1958, describe the swimbladder of the toadfish.) He finds the choroid rete to differ from that of the toadfish swimbladder rete in several respects. (1) The venous channels are not discrete, straight vessels parallel to the arterial capillaries, but appear to form a labyrinthine system of sinusoidal vessels which often seem to surround the arterial capillaries. Wolley and Copeland (1970) find that venous channels in the teleost rete may also coalesce. (2) The differences in thickness of the walls of the two classes of vessels is very slight in striking contrast to the swimbladder rete. (3) The endothelial cell junctions which are simple in the swimbladder and show prominent desmosomes, are more imbricated and inter-

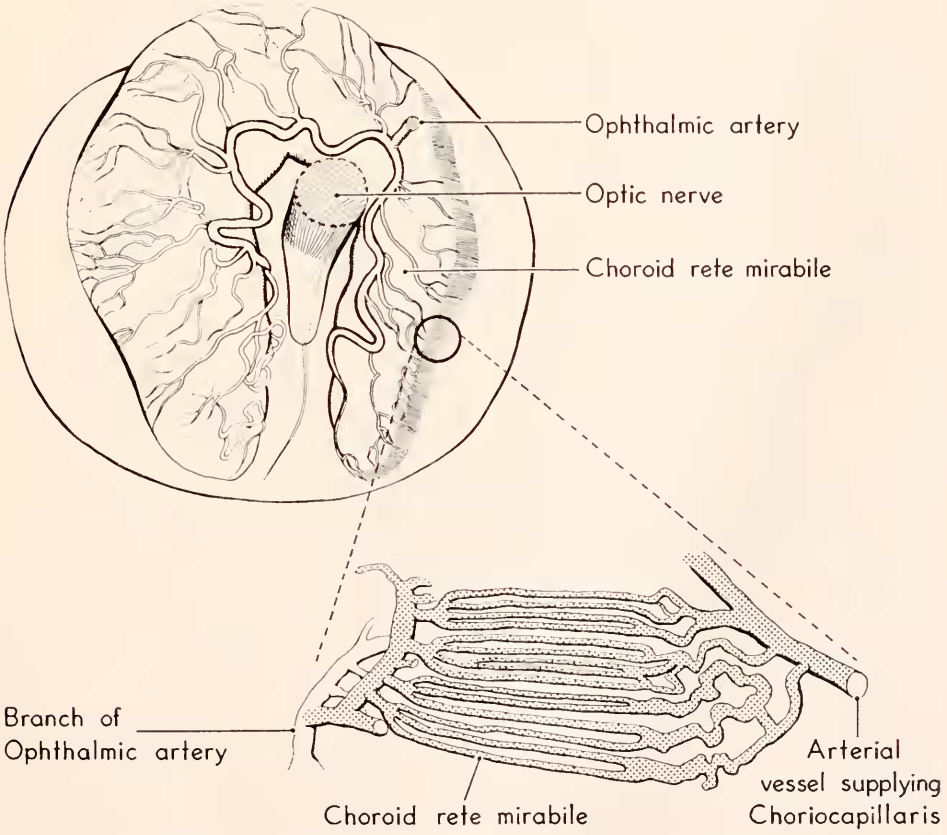

FIGURE 6. Choroid rete mirabile of the eye of *Amia*. Right eye viewed from inside the orbit. Only arterial structures are shown. The insert shows the arterial capillaries of the choroid rete at greater magnification.

digitated in the choroid rete and have no desmosomes. (In this respect they resemble mammalian capillaries more than do the toadfish capillaries.) (4) A particulate component of the plasma is rather consistently about five-fold more concentrated in the venous than in the arterial vessels.

It is of interest to compare the choroid and swimbladder retia mirabilia. The general features of the choroid rete of teleosts and of *Amia* are similar to those of the swimbladder rete mirabile but differences are noted: (1) The composition of the blood reaching the two retia may differ (Wittenberg and Haedrich, 1974) since blood comes to the choroid rete via the pseudobranch and to the swimbladder

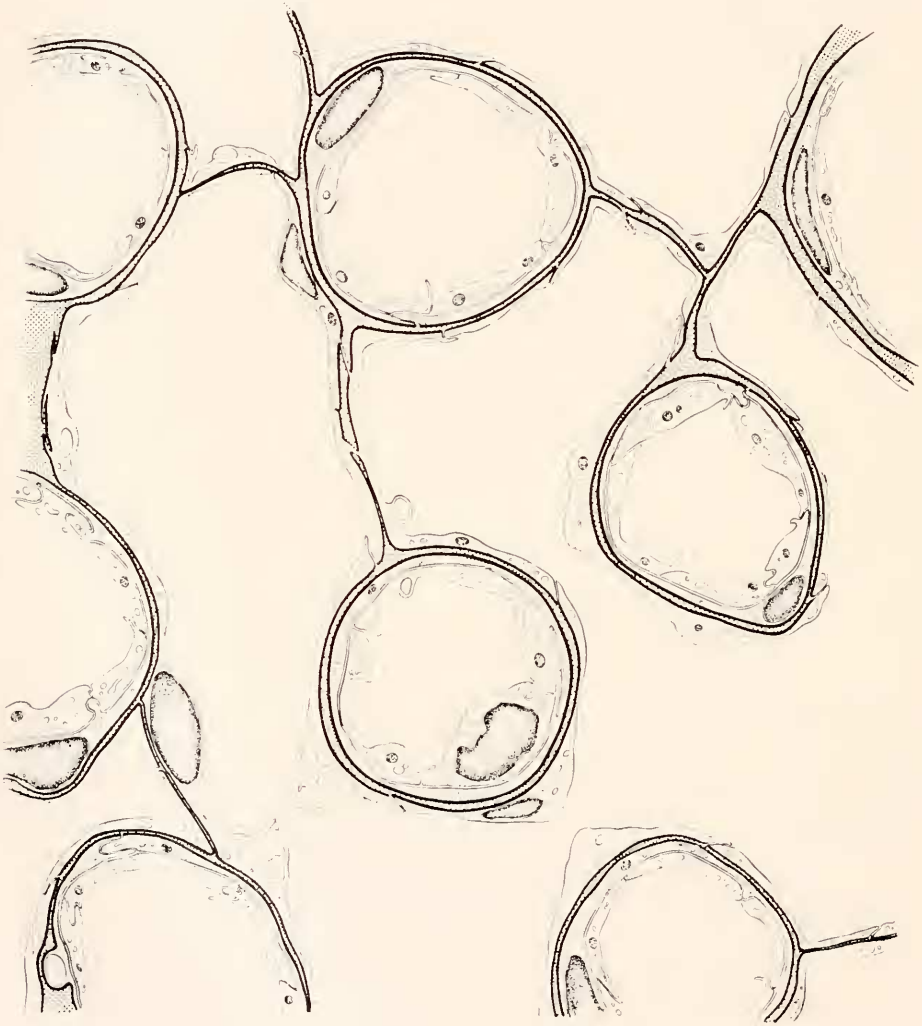

FIGURE 7. Diagrammatic cross-sections of the choroid rete of *Amia*, above, and the swimbladder rete of the toadfish, opposite; drawn from electron micrographs by Dr. Don W. Fawcett.

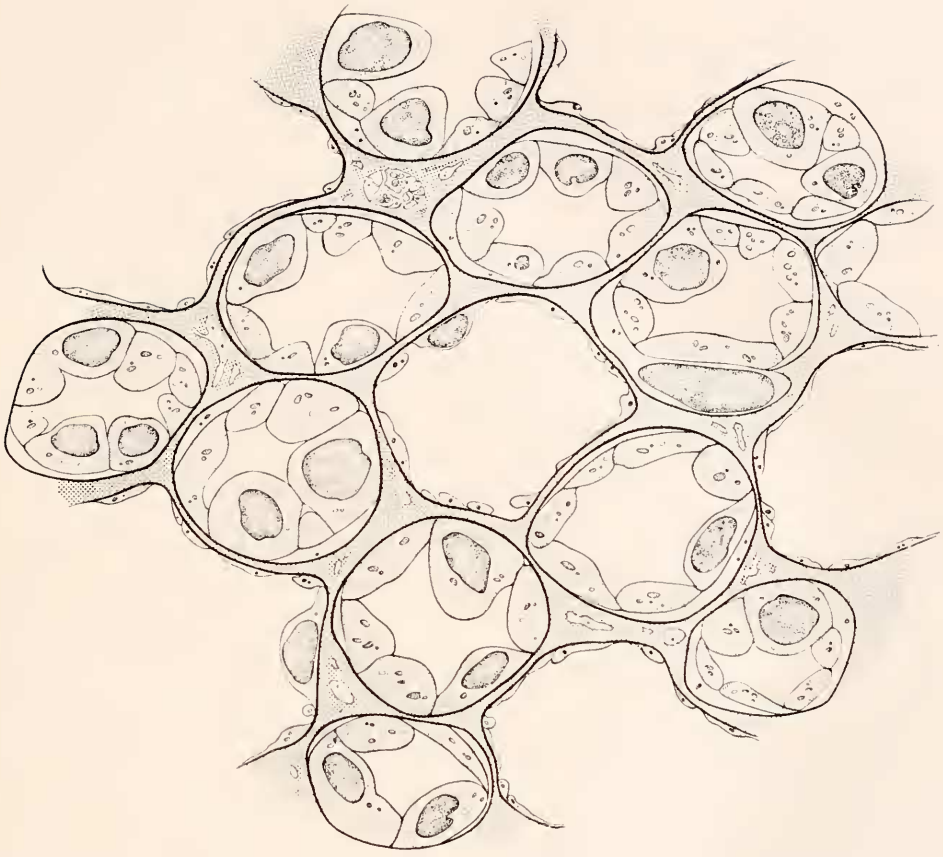

FIGURE 7. (*Continued*)

rete from the general circulation. (2) The difference in thickness of the walls of arterial and venous capillaries is very slight in the choroid rete of *Amia*. In contrast the arterial capillaries of the swimbladder rete are distinguished from those of the venous capillaries in that they are exceptionally thick and their cytoplasm contains a great abundance of smooth-surfaced vesicles (Fawcett and Wittenberg, 1959; Dorn, 1961; Fahlén, 1967; Jasiński and Kilarski, 1971). This points to some intracellular process more developed in the swimbladder than in the choroid rete. (3) The capillaries of the choroid rete are sharply differentiated into proximal and distal portions of differing structure; the capillaries of the swimbladder rete appear the same throughout. Although it is reasonable to consider that the general pattern of the physico-chemical mechanism by which large oxygen pressures are established is the same in the choroid and swimbladder retia, the structural differences are sufficient to suggest substantial differences in detail.

Krogh, in his celebrated book *The Anatomy and Physiology of the Capillaries* (1922), reports quantitative measurements of the capillaries of the rete mirabile of the swimbladder of an eel, and makes a plea that others should take up the study

of quantitative anatomy. We begin by estimating the distance that oxygen must diffuse to supply the retina. If in fact the function of the system comprised by the choroid rete mirabile, the choriocapillaris and the pigment cell layer of the retina is to supply the retina with oxygen, we may enquire whether there is any unusual impediment to be overcome in the flow of oxygen from the capillaries of the choriocapillaris to the sites of oxygen consumption. The mitochondria of the photoreceptor cells are for the most part aggregated into a special structure, the spheroid, lying between the photoreceptive rod and cone outer segments and the respective cell soma. The distance from the inner surface of the choriocapillaris to the spheroid was measured in 26 representative species including carp, trout and 24 species of marine teleosts. The average distance from the inner surface of the choriocapillaris to the spheroid is 106 micrometers (range 78 to 170 micrometers). Although many teleosts have blood vessels (the retinal vessels) displayed on the inner surface of the retina, many do not, and all of the cells of the retina of these latter must depend on the choriocapillaris for their supply of oxygen. The average retinal thickness for the same sample was 360 micrometers (range 240 to 550 micrometers). These distances, across which diffusion must occur in the fish retina, are large. Compare for instance red muscle in which the diffusion path, one half the intercapillary distance, does not exceed 20 micrometers (Wittenberg, 1970). On the other hand, the distance which oxygen must diffuse in the primate retina is larger than in many other tissues. The retinal circulation of the primate penetrates from the inner face of the retina only as far as the outer plexiform layer; the layers corresponding to the photoreceptors are avascular and must depend for their oxygen on diffusion from the retinal and choroidal circulations (Polyak, 1957); the diffusion path may be of the order of 60 micrometers (Polyak, 1941). We note that the diffusion path in the fish retina is very long indeed and exceeds by more than six fold the diffusion path in the primate retina.

In Table IV we compare the dimensions of two swimbladder retia and two choroid retia supplying blood to capillary beds of about the same area. The task of the swimbladder rete is to maintain a volume of gas in the lumen in the face of the ambient pressure. The capillaries are of about the same length in two shallow-living species, bluefish and conger, whose patterns of gas secretion are quite different (Wittenberg, Schwend and Wittenberg, 1964; Fänge and Wittenberg, 1958). Marshall (1960), who has studied the swimbladders of deep sea fishes, notes that the length of the capillaries of the swimbladder rete increases with the depth at which the fish lives; the capillaries in abyssal fish may be 2.5 cm long; while the number of capillaries is proportional to the size of the fish. Following Marshall, we consider the eel, Table IV, as an example of a deep sea fish, albeit captured from shallow water. The retial capillaries are long, but the number of capillaries, and by that token, the cross-section area of the rete are not great.

The teleost retina consumes oxygen vigorously (Hoffert and Fromm, 1972), and the question arises how the choroid rete is adapted to meet this demand. A measure of the demand is given by the data of Hoffert (private communication) who finds that the retina of trout eyes (fish weight 98 gram, 15° C) consume 40.8 μliter O_2 per hour per eye, which is equivalent to 9.02 μliter O_2 per hour per cm^2 retinal area. From the data in Table IV we estimate that each cm^2 of retina is served by approximately 16–25 million arterial capillaries of the choroid rete.

The capillaries of the choroid rete are relatively short; the average length in teleosts is 0.14 cm (range of 86 teleosts, 0.052 to 0.18 cm; standard deviation 0.42). Although the fish in our sample weighed from a few grams to over 200 kilograms, the length of the capillaries was remarkably similar in all, and capillary length showed no correlation with body size. The average length of the capillaries of the rete of *Amia* was 0.21 cm. Dissection of the eyes of many species reveals that the increased demand accompanying larger size is met by a proliferation of a large number of short capillaries, so that the choroid rete mirabile in very large fish becomes a veritable sheet of tissue with capillaries arrayed normal to the plane of the sheet. This is exemplified by data on the tuna eye, Table IV, in which the rete contains 7.94 million arterial and 8.14 million venous capillaries, sixteen million in all, arrayed in parallel and each only 0.18 cm long. Their aggregate length is 2900 meters, and, as Krogh (1922) points out, this arrangement allows a very large surface for the exchange of substances to be provided within a small volume.

We conclude that the choroid rete mirabile is adapted to deliver a large flow of oxygen to the retina at the relatively low pressure required to overcome the barrier imposed by the long diffusion path in the retina.

We turn to a discussion of the role which movements of water may play in the physiology of the choroid and swimbladder retia. Electron micrographs of the choroid rete of *Amia calva*, mentioned above, and also of the swimbladder rete of *Opsanus tau*, reveal significant differences in the concentration of the plasma in the lumen of the arterial and venous capillaries, suggesting that there may be a considerable flux of water from one set of capillaries to the other. The direction of these fluxes is not established by the electron micrographs because we do not know from where in the rete the samples were taken. We now enquire whether substantial movements of water between the two classes of vessels of the rete mirabile may not be a part of the normal operation of the counter-current system secreting oxygen, in this instance into the swimbladder of the eel, *Anguilla vulgaris*.

Data bearing on this point are available from the elegant experiments of Steen (1963), who analyzed blood drawn from each of the blood vessels of the gas-secreting complex of the eel swimbladder during states of sustained oxygen secretion. The vessels from which blood was drawn are indicated as A, B, C and D in Figure 1. We focus attention on lactic acid. During gas secretion, aerobic glycolysis by the cells of the gas gland generates lactic acid which enters the blood flowing from points B to C. This is at once apparent from inspection of Steen's data, since the concentration of lactic acid is, in every experiment, much greater in blood leaving the gland at C than in blood entering at B. Steen, in interpreting his data, tacitly assumes that water neither enters nor leaves the capillaries of the rete. On that basis material balance of lactic acid is not achieved. The difference in concentration between the inflowing and outflowing blood (C_D-C_A) does not equal the difference across the gas gland, nor is the apparent loss of lactic acid in the venous capillary matched by an equivalent gain in the arterial vessels. This is not satisfactory. On the other hand, an internally consistent interpretation of the data emerges if we assume that: (1) Water may move between the two classes of capillary in the rete. (2) The sustained state of oxygen secretion studied by Steen was in fact a steady state. And, (3) lactic acid is not destroyed in the rete. The magnitude and direction of the water fluxes may be deduced.

All calculations are per unit time. Consider a unit volume of blood, V, entering the complex at A and containing X millimoles of lactic acid. The concentration of lactic acid at A, C_A, will be:

$$C_A = \frac{X}{V} \tag{1}$$

Consider that an amount of lactic acid, Y, passes between the arterial and venous capillaries. Considered also that a volume of water, ΔV, moves between the arterial and venous capillaries. The concentration of lactic acid at B, C_B, will be:

$$C_B = \frac{X - Y}{V - \Delta V} \tag{2}$$

There is no opportunity for water exchange in the capillaries of the gas gland. We note that an amount of lactic acid, Z, is added to the blood by glycolysis occurring in the cells of the gas gland. The concentration of lactic acid at point C, C_C, therefore will be:

$$C_C = \frac{X - Y + Z}{V - \Delta V} \tag{3}$$

Finally, since at steady state the volume of fluid leaving is equal to the volume entering, we have for the concentration of lactic acid at D, C_D:

$$C_D = \frac{X + Z}{V} \tag{4}$$

At steady state the amount of lactic acid added to the blood by the cells of the gas gland, Z, is equal to the added lactic acid, Z, leaving the rete at point D. Combining expressions 1 and 4, and 2 and 3, we have two expressions in Z, which do not involve Y. Combining these, we express ΔV in terms of V and of the experimentally measured lactic acid concentrations:

$$\frac{\Delta V}{V} = 1 - \left(\frac{C_D - C_A}{C_C - C_B}\right) \tag{5}$$

Here $\Delta V/V$ is the fraction of the total blood flow which passes from arterial to venous capillaries.

We obtain two expressions for Y/V, the amount of lactic acid moving between the arterial and venous capillaries per unit blood flow, as a function of $\Delta V/V$ and of the lactic acid concentrations at points A and B or at points C and D.

$$\left(\frac{Y}{V}\right)_{A,B} = C_A + C_B\left(\frac{\Delta V}{V} - 1\right) \tag{6}$$

$$\left(\frac{Y}{V}\right)_{C,D} = C_D + C_C\left(\frac{\Delta V}{V} - 1\right) \tag{7}$$

Steen's data for the lactic acid concentrations in the vessels of the gas secreting complex, together with the derived values $\Delta V/V$, $(Y/V)_{A,B}$ and $(Y/V)_{C,D}$ are presented in Table V. The agreement in the values of $(Y/V)_{A,B}$ and $(Y/V)_{C,D}$, respectively lactic acid leaving the arterial and entering the venous capillaries,

TABLE V

Calculated movement of water from arterial to venous capillaries of the eel swimbladder rete mirabile. The concentrations of lactic acid in the blood drawn from the retial vessels are those determined by Steen (1963). Blood vessels A, B, C, D are indicated in Figure 1.

Experiment	Lactic acid concentration in blood of vessels A, B, C, D				Derived values		
	C_A	C_B	C_C	C_D	$\left(\frac{V}{\bar{V}}\right)_{A,B}$	$\left(\frac{V}{\bar{V}}\right)_{C,D}$	$\frac{\Delta V}{\bar{V}}$
	mM	mM	mM	mM	mM	mM	
A	2.7	3.7	7.8	3.4	2.1	2.1	0.83
B	9.8	14.9	20.9	13.1	1.6	1.6	0.45
C	3.2	4.4	10.9	5.1	1.9	1.9	0.71
D	4.4	5.7	11.1	5.9	2.8	2.8	0.72
E	6.2	9.1	16.9	9.9	1.9	1.9	0.53
F	8.8	8.9	12.3	8.9			
G	1.6	5.0	10.2	6.7	−3.4	−3.4	0.02
H	3.5	8.9	16.1	8.0	−2.0	−2.0	0.38

shows that calculations based on the assumption of water movement are internally consistent.

The value of $\Delta V/V$ is large in six of Steen's eight experiments, showing a net water flow from arterial to venous capillaries of the rete. Actually, in light of the fact that the hematocrit in these experiments ranged from 37 to 50 per cent, the calculated value of ΔV is improbably large, no doubt reflecting a systematic error.

The direction of movement of lactic acid is of some interest. A flux of lactic acid from a region of high concentration, the venous capillaries, to a region of lower concentration, the arterial capillaries, might be expected if the capillary walls are permeable to lactic acid. In Steen's experiment G the water flow is negligible, in his experiment H it is small; in these two experiments we note a flow of lactic acid down the concentration gradient. In each of his other experiments lactic acid apparently accompanies the moving water from arterial to venous capillaries.

Kuhn and his collaborators have proposed that oxygen concentration by the swimbladder rete is achieved by means of a counter-current multiplication mechanism (*e.g.*, Kuhn, Ramel, Kuhn and Marti, 1963; for a simple statement of Kuhn's hypothesis see Wittenberg, Schwend and Wittenberg, 1964). The operation of this mechanism requires that the lactic acid concentration in the outflowing venous vessels of the rete exceed that in the arterial vessels. Steen's data demonstrate that this requirement is in fact met in the working rete. We ask whether the movement of water from arterial to venous vessels does not oppose the establishment of the required large concentration of lactic acid in the venous vessels. The calculated quantity, $\Delta V/V$, represents a net movement of water along the whole length of the retial capillaries. If the bulk of this water movement takes place near the distal (swimbladder) pole of the rete the crucial concentration of lactic acid in the venous capillaries would be much dissipated. If, on the other hand, the bulk of the water movement occurs near the proximal (cardiac) pole of the rete, by increasing the concentration of solutes in the distal part of the rete, it might have the effect of enhancing the operation of the counter-current multiplication system.

We thank Dr. Charles Wheeler, Dr. D. Eugene Copeland, Mr. William A. Lemberger, and the staff of the Supply Department, Marine Biological Laboratory, Woods Hole, for their help in obtaining the animals used in this study. We also thank Drs. D. Eugene Copeland, Richard L. Haedrich and Francis G. Carey for continuing discussions, and Dr. J. Russell Hoffert for making available the results of his experiment prior to publication. Particular thanks are due Dr. Don W. Fawcett for permission to present his unpublished drawings based on his electron micrographs of the retial capillaries and to Mrs. Julia F. Child who did the other drawings. The skilled assistance of Miss M. J. Schwend is gratefully acknowledged.

Summary

Very large pressures of oxygen are found in the vitreous humor close to the retina of many teleosts and of the holostean, *Amia*. Elevated pressures are not found in species lacking a choroid rete mirabile. In species having choroid retia, the observed oxygen pressure roughly parallels the extent to which the rete is developed. On this evidence we suggest that the choroid rete mirabile, acting in concert with the pigment cell layer of the retina, plays an essential part in establishing the large oxygen pressure at the retina.

The choroid rete mirabile of the holostean, *Amia calva*, is essentially similar to the teleost choroid rete, from which it differs only in details of structure.

The capillaries of the counter-current organ, the choroid rete, are short, compared to the capillaries of the swimbladder rete, and are very nearly the same length in all the fishes examined, independent of the size of the fish, which ranged from tens of grams to hundreds of kilograms. The increased demand accompanying larger size is met by increasing the number of capillaries. We conclude that the structure of the choroid rete mirabile is adapted to supply a large flux of oxygen toward the retina at a relatively low pressure.

The general features of the structure of the choroid rete mirabile are largely similar to those of the swimbladder rete mirabile, but the two retia differ sufficiently to suggest that the physico-chemical mechanisms by which they build up large oxygen pressures may also differ in detail.

It is suggested that movements of water between inflowing and outflowing capillaries of the rete mirabile may play an important role in the mechanism by which oxygen is secreted into the eye and into the swimbladder.

LITERATURE CITED

Albers, J. A. A., 1806. Über das Auge des Kabeljau Gadus morhua und die Schwimmblase der Seeschwalbe, Trigla hirundo. *Göttingen gelehrte Anzeiger*, 2: 681–682.

Allen, W. F., 1905. The blood vascular system of the Loricati, the mail-cheeked fishes. *Proc. Washington Acad. Sci.*, 7: 27–157.

Allen, W. F., 1949. Blood vascular system of the eye of a deep water fish (*Ophiodon elongatus*) considered as a pressure mechanism. *Anat. Rec.*, **103**: 205–212.

Alm, A. and A. Bill, 1972. The oxygen supply to the retina, I. Effect of changes in intraocular and arterial blood pressures, and in arterial pO_2 and pCO_2 on the oxygen tension in the vitreous body of the cat. *Acta Physiol. Scand.*, **84**: 261–274.

Barnett, C. H., 1951. The structure and function of the choroidal gland of teleostean fish. *J. Anat.*, **85**: 113–119.

Denton, E. J., J. D. Liddicoat and D. W. Taylor, 1970. Impermeable "silvery" layers in fish. *J. Physiol.*, **207**: 24P–25P.

Dorn, E., 1961. Über den Feinbau der Schwimmblase von *Anguilla vulgaris* L. *Z. Zellforsch.*, **55**: 849–912.

Fahlén, G., 1967. Morphology of the gas bladder of *Coregonus lavaretus* L. *Acta Universitatis Lundensis*, **28**: 1–37.

Fairbanks, M. B., J. R. Hoffert and P. O. Fromm, 1969. The dependence of the oxygen-concentrating mechanism of the teleost eye (*Salmo gairdneri*) on the enzyme carbonic anhydrase. *J. Gen. Physiol.*, **54**: 203–211.

Fänge, R. and J. B. Wittenberg, 1958. The swimbladder of the toadfish (*Opsanus tau* L.). *Biol. Bull.*, **115**: 172–179.

Fawcett, D. and J. Wittenberg, 1959. The fine structure of capillaries in the rete mirabile of the swimbladder of *Opsanus tau*. *Anat. Rec.*, **133**: 274.

Harden Jones, F. R. and N. B. Marshall, 1953. The structure and functions of the teleostean swimbladder. *Biol. Rev.*, **28**: 16–83.

Hoffert, J. R. and P. O. Fromm, 1972. Teleost retinal metabolism as affected by acetazolamide. *Proc. Soc. Exp. Biol. Med.*, **139**: 1060–1064.

Jacobi, K. W. and F. Driest, 1965. Sauerstoffbestimmungen im Glaskörper des lebenden Auges. *Berichte Deutsche Ophthalmologische Gesellschafte*, **67**: 193–198.

Jasiński, A. and W. Kilarski, 1971. Capillaries in the rete mirabile and in the gas gland of the swimbladder in fishes, *Perca fluviatilis* L. and *Misgurnus fossilis* L. *Acta Anat.*, **78**: 210–223.

Jones, T. W., 1838. On the so-called choroid gland or choroid muscle of the fish's eye. *London Med. Gaz.*, **21**: 650–652.

Krogh, A., 1922. *The Anatomy and Physiology of the Capillaries*. Yale University Press, New Haven, Connecticut, 276 pp

Kuhn, W., A. Ramel, H. J. Kuhn and E. Marti, 1963. The filling mechanism of the swimbladder. Generation of high gas pressures through countercurrent multiplication. *Experientia*, **19**: 497–511.

Linthicum, D. S. and F. G. Carey, 1972. Regulation of brain and eye temperatures of the bluefin tuna. *Comp. Biochem. Physiol.*, **43A**: 425–433.

Marshall, N. B., 1960. Swimbladder structure of deep-sea fishes in relation to their systematics and biology. *Discovery Reports*, **31**: 1–122.

Müller, J., 1839. Vergleichende Anatomie der Myxinoiden. III. Über das Gefässystem der Choroidalkörpers in Auge der Knochenfische. *Abhandlungen der Preussischen Akademie der Wissenschaften Berlin*, **1839**: 254–261.

Nelson, G. J., 1969a. Origin and diversification of teleostean fishes. *Ann. New York Acad. Sci.*, **167**: 18–30.

Nelson, G. J., 1969b. Gill arches and phylogeny of some fishes, with notes on the classification of vertebrates. *Bull. Amer. Mus. Natur. Hist. (New York)*, **147**: 475–552.

Owen, R., 1836. *Physiological catalogue of the Hunterian Museum*, Vol. 3. Royal College of Surgeons, London, 1656.

Polyak, S. L., 1941. *The Retina*. University of Chicago Press, Chicago, Illinois, 607 pp.

Polyak, D. L., 1957. *The Vertebrate Visual System*. University of Chicago Press, Chicago, Illinois, 1156 pp.

Steen, J. B., 1963. The physiology of the swimbladder in the eel *Anguilla vulgaris*. III. The mechanisms of gas secretion. *Acta Physiol. Scand.*, **59**: 221–241.

Stevens, E. D. and D. J. Randall, 1967. Changes of gas concentrations in blood and water during moderate swimming activity in rainbow trout. *J. Exp. Biol.*, **46**: 329–337.

Wittenberg, J. B., 1970. Myoglobin-facilitated oxygen diffusion: role of myoglobin in oxygen entry into muscle. *Physiol. Rev.*, **50**: 559–636.

Wittenberg, J. B., M. J. Schwend and B. A. Wittenberg, 1964. The secretion of oxygen into the swimbladder of fish. III. The role of carbon dioxide. *J. Gen. Physiol.*, **48**: 337–355.

Wittenberg, J. B. and R. L. Haedrich, 1974. The choroid rete mirabile of the fish eye. II. Distribution and relation to the pseudobranch. *Biol. Bull.*, **146**: 137–156.

Wittenberg, J. B. and B. A. Wittenberg, 1962. Active secretion of oxygen into the eye of fish. *Nature*, **194**: 106–107.

Wolley, R. C. and D. E. Copeland, 1970. The choroid rete of the scup, *Stenotomus versicolor,* and striped bass, *Roccus saxatilus. Biol. Bull.,* **139**: 444.

Woodland, W. N. F., 1911. On the structure and function of the gas glands and retia mirabilia associated with the gas bladder of some teleostean fishes, with notes on the teleost pancreas. *Proc. Zool. Soc. London,* **1911**: 183–248.

Note added in proof: The following article has come to our attention since this manuscript was submitted. Steen, J. B., 1970. The rete mirabile and a note on its diffusion characteristics. Pages 394–397 in C. Crone and N. A. Lassen, Eds., *Capillary Permeability, Proceedings of the Alfred Benzon Symposium II.* Academic Press, New York.